FORM REGULATION IN ZOÖTHAMNIUM ALTERNANS

F. M. SUMMERS

(From Bard College, Columbia University)

Introduction

Interest in morphogenetic studies has long centered about the part-whole relationships obtaining throughout the formative period in organic development. In so far as a system comprising several parts is concerned, the term *organization* implies the existence of integrating factors that condition to some extent the limits and direction of regional specialization. A remarkable number of investigations on metazoan organizers have already demonstrated the importance of extrinsic factors upon determination in specific parts. It was felt that additional information about these factors could be gained by applying operative techniques to an animal type in which, presumably, the interrelationships have not attained so great a degree of complexity.

The principal endeavor of this work was to investigate some of the qualitative and quantitative aspects of growth and differentiation in *Zoöthamnium* colonies, regulating after the removal of actively growing (distal) parts, and to compare regulative behavior with the normal development already described by Fauré-Fremiet (1930) and Summers (1938). The results have made it possible to offer a rough map of potencies and prospective values of individual cells at various positions in the colonial pattern and to indicate some of the changes in the expression of inherent potencies which may be induced by experimental means.

It is a pleasure to thank Professor L. R. Cleveland of Harvard University for extending to the author the privileges of his laboratory during January, 1937.

Materials and Methods

The materials and techniques used in this study are similar to those described in the previous paper and need not be repeated here. The only additional detail pertains to the method of shearing the stalk. Two fine scalpels made from No. 9 sewing needles were used for the purpose. One was brought to rest against the surface of the stalk and the other sheared against it in scissors fashion.

The plan of attack is by no means new, but the type of organization

dealt with seems to promise a fresh approach to the current problems of form determination. A colony of *Zoöthamnium alternans* is admirably adapted to work of this kind by virtue of the regularity and precision with which the characteristic colonial pattern develops. The alternating arrangement of the branches and cells makes it comparatively easy to follow the history of any one cell throughout the course of its development for evidences of growth, division, or differentiation. The spatial relationship of the cells minimizes to a great extent some of the factors so difficult to evaluate for compact tissues. Crowding effects such as mutual contact, pressure, etc. (Peebles, 1931) are of no great concern here. Then, too, the separated cells are uniformly bathed by an almost constant medium, filtered sea water. Physiological relations between them are effected through a well-defined channel, the stalk with its neuro-muscular cord (Fauré-Fremiet's "cordon central"), rather than through the general expanse of juxtaposed cell membranes.

Regulative Development

Standards of Judgment.—Colonies maintained for several days on slides are apt to be attacked by internal parasites or covered by plant growths of one kind or another, especially in the basal regions. When operations are made the axial growth of a colony is retarded for an average of 23.1 hours pending the formation of a new terminal macrozoöid. It is during this period of arrested development that adverse environmental conditions are liable to bring about an incapacitation or loss of important zoöids before a decision relative to the success of the operation can be reached. A small proportion of the successful operations shown in Tables I and II do not appear in subsequent tables because they were destroyed or abandoned after indubitable signs of new terminal macrozoöid differentiation had appeared but before descendants were produced. In the absence of a regenerate, the responses were recorded only when all of the structural characteristics of the new terminal macrozoöid were established and, in addition, the "activated" branch developed an anterior flexure. In consequence of the stalk curvature the new terminal macrozoöid assumes the apical position upon an anteriorly directed axial stalk. The point of curvature marks the node (Figs. 3 and 5) at which the stalk suddenly increases to a diameter approaching that of the original axis.

Regenerative responses were arbitrarily called negative only when one of the following conditions were realized: (*a*) there was no activity for at least 48 hours; (*b*) in the event that mitotic activity continued for a generation or two, a minimum of 72 hours was allowed for signs

of regulatory activity; (*c*) when the terminal branch zoöid in the line of succession metamorphosed into a migrating zoöid of some kind. The thirty-seven negative cases shown in Tables I and II were maintained for a mean time of 94.3 hours after the last division, with extremes of 52 to 212 hours.

TABLE I

LEVEL OF OPERATION	PARTS CUT AWAY								
	TM.+0	TM.+1	TM.+2	TM.+3	TM.+4	TM.+5	TM.+6	TM.+7	Total
Q	1								1
P	(1)	(2)		(1)					(4)
O	2 (1)	1 (1)	(1)	(1)					3 (4)
N		(1)							(1)
M	1 (1)	(1)							1 (2)
L	(1)	(1)							(2)
K	1		(1)	(1)					1 (2)
J	2 (1)								2 (1)
I		1	1	1	(1)				3 (1)
H	1	(1)				(1)			1 (2)
G	3 (1)	1 (1)	(1)	(1)					4 (4)
F	4	1							5
E	1	1		1					3
D	3	2							5
C	6	4		1	1				12
B	3 (1)	4 (1)	2		1		(1)		10 (3)
A	9 (6)	6 (3)	6	2 (2)	1	1	1	1	27 (11)
Total	37 (13)	21 (12)	9 (3)	5 (6)	3 (1)	1 (1)	1 (1)	1	78 (37)

The distribution of regulative responses in 115 operated colonies summarized according to the number of apical branches cut away: *T.M.* + 0 = only the terminal macrozoöid removed; *T.M.* + 1, *T.M.* + 2, etc. = terminal macrozoöid plus one, two, or more branches removed. The letters in the left-hand column designate the branches from which the regenerates arose. The numbers of cases in which no response occurred are shown in parentheses. The first responses of the successively operated colonies are included. No attempt has been made to indicate the number of zoöids on the branches dissected away; it may be estimated by referring to Fig. 1 in the previous paper.

All colonies were abandoned when the important zoöids appeared to be unhealthy.

Adjustment of Descriptive Notation.—An arbitrary departure from the standard notation (see Fauré-Fremiet, 1930; or Summers, 1938) seems to be advisable in view of the complications arising from the

designation of generations produced by the secondary zoöid near the point of origin of the new terminal macrozoöid. To illustrate: if the new terminal macrozoöid differentiates directly from $2a^1$, then the secondary zoöid $2a^2$ continues to generate zoöids of the branch A, viz. $2a^{21}$, $2a^{22}$; $2a^{211}$, $2a^{212}$, etc., which complicates a system already difficult to summarize briefly.

At this point it is proposed to adjust the terminology so that the new terminal macrozoöid (actually $2a^1$) corresponds in position to the

TABLE II

		ORIGIN OF NEW TERMINAL MACROZOOID										
		IX	IX′	Ix	2x′	2x²	3x′	4x′	4x²	5x′	6x′	Total
LEVEL OF OPERATION	Q			1								1
	P			(1)	(1)		(1)			(1)		(4)
	O	2			1 (1)		(2)	(1)				3 (4)
	N						(1)					(1)
	M			(1)	1		(1)					1 (2)
	L				(1)					(1)		(2)
	K			1				(1)			(1)	1 (2)
	J			2	(1)							2 (1)
	I	1	1			1			(1)			3 (1)
	H						1		(1)	(1)		1 (2)
	G		1	2	1 (2)	(1)				(1)		4 (4)
	F			2	2	1						5
	E	1			1			1				3
	D			1	3			1				5
	C			8	3		1					12
	B			5 (2)	2	1	1	1	(1)			10 (3)
	A	1 (1)	(1)	15 (7)	5	(1)	5 (1)	1				27 (11)
Total		5 (1)	2 (1)	37 (11)	19 (6)	3 (2)	8 (6)	4 (2)	(3)	(4)	(1)	78 (37)

Lateral distribution of operations. This table summarizes the data from Table I according to regulation by branch generations irrespective of the amount of colony cut away. The left-hand column represents the branches from which the regenerates developed; headings indicate the position and generation of the zoöids which produced the new terminal macrozoöid. The negative responses are shown in parentheses.

original terminal macrozoöid (*T. M.* No. 1), but is distinguished from it by a prime (′) symbol. The lateral branch zoöid $2a^2$ becomes A', the first zoöid of a new branch at the original A level (Fig. 1).

This adjustment is valid if subsequent products are to be treated descriptively as new primary branches rather than as branches of the second order on the first branch below the cut. This is nothing more than a manipulation of terms to facilitate comparison of normal and regulating colonies.

Trauma.—For a number of reasons it appears probable that traumatic shock effects are not significant factors in post-operative adjustments of colonial form. Cells adjacent to the cut areas and elsewhere soon expand and feed as before. Processes of mitosis or

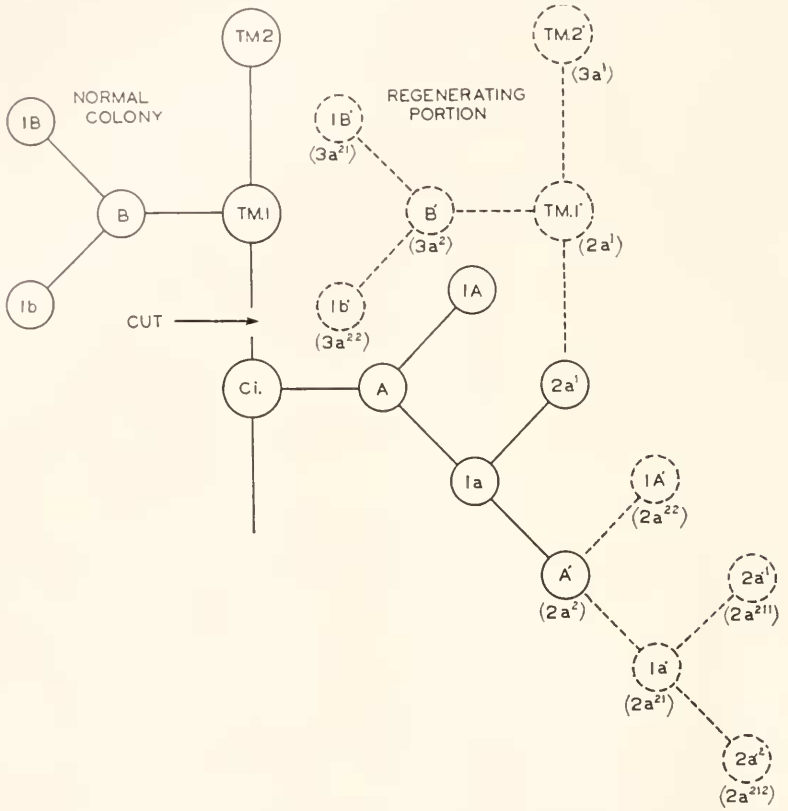

FIG. 1. Diagram of regulative development. The revised notation for designating cell lineage in regulating portions is indicated within the circles. The symbols in parentheses under the circles illustrate how cumbersome the conventional terminology would soon become if applied to the regenerates. The diagram shows the lineage of a regenerate from branch *A*. If the principal axis is severed between branches *A* and *B* at a time when there are three cells on branch *A*, the regenerate usually arises from the terminal branch zoöid $2a^1$ as illustrated. The sub-terminal zoöid ($2a^2$) assumes the terminal branch position *A'* and continues to generate branch cells. The new branch *B'* is produced by the first division of the new terminal macrozoöid *T.M.* 1'. The parts produced after cutting are drawn with broken lines.

differentiation, in progress at the time of operation, continue without perceptible interruption. Or these processes may begin at varying intervals after cutting, in any of the branch zoöids, except that which becomes the new terminal macrozoöid. Furthermore, when there is

no regulation, or when the median microzoöid divides one or more times before the new terminal macrozoöid is recognizable, the first branch below the cut continues to generate common zoöids as before. Relative to division rates, the available data indicate that, exclusive of the one which bears the presumptive terminal macrozoöid, branch growth is not perceptibly altered after cutting. As a rule there is a lag in the development of the activated branch pending the differentiation of a new growing point.

The only effects of mechanical disturbance are evidenced for a very short time after cutting by a state of irritability during which the contraction of decapitated colonies is frequent, irregular, and sometimes tetanic. But normal overt behavior is resumed within a few moments when the stalks are shorn cleanly at internodal points. Operations were considered acceptable only when the normal reactions were regained within a relatively short time. Cases where only the neuro-muscular cord of the stalk was injured are to be treated in another section.

Distribution of Operations.—A general résumé of the experimental results in terms of initial regulative responses in *Zoöthamnium alternans* is given in Tables I and II. Of the 144 protocols at hand (acceptable operations), 78 yielded positive responses and 37 were negative; the remainder were inconclusive according to the standards chosen and are omitted in the digests.

Table I summarizes the responses to various types of cuts made at the several levels along the principal axis irrespective of the number of generations on the regulating branches. In Table II the same protocols are tabulated according to regulative responses by the various branch generations without regard to the number of branches or zoöids removed. In this table the negative cases show only the zoöids which were expected to reconstitute the axial growing point; some of these, failing to regulate, continued to develop laterally without further differentiation.

The symbols X and x are used to indicate generations on a generalized branch (Summers, 1938). With reference to a specific branch, e.g. branch D, the symbol $1x$ refers to the microzoöid $1d$, and $1X$ means axial microzoöid $1D$.

Simple Cut-offs.—In general when the terminal macrozoöid was cut off, the terminal cell of the first branch below the cut differentiated into a new, well-defined terminal macrozoöid whose first and subsequent divisions proliferated the alternating median microzoöids (initial branch cells) of the regenerate. Some operations were made at a time when a single cell, the median microzoöid X, represented the

adjacent rudimentary branch. In all such instances recorded at least one division followed without perceptible delay, thus producing an axial microzoöid 1*X* and the presumptive terminal macrozoöid 1*x*. More frequently the removal of a terminal macrozoöid left two or three cells on the last branch (Fig. 1). Simple cut-offs leaving this branch with more than three cells were rarely possible for the reason that a division of the terminal macrozoöid usually preceded the third division on the adjacent branch.

There were a number of cases where, after an operation, the appointed branch continued to develop at a normal rate for one or more

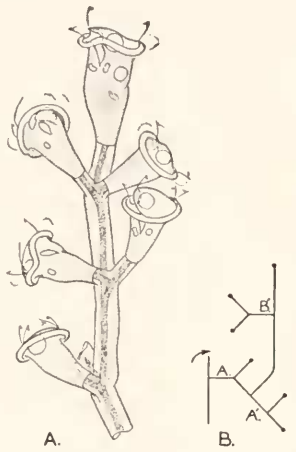

FIG. 2. *A*. Colony *3II3-5* drawn approximately 53 hours after the operation. The apical cell was destroyed at the two-cell stage of development. The remaining microzoöid *A* divided twice before the new *T.M.* differentiated from $2a^1$. Its first division produced the initial cell of the branch *B'*. The alternate daughter ($2a^2$) extended the original branch, and the axial microzoöid 1*A* persisted without further change. × 250.

B. Schematic representation of the resulting growth.

generations before another terminal macrozoöid differentiated (Fig. 2). In approximately 12 per cent of the regulating colonies two divisions followed the operation, within normal time limits, before indications of a new terminal macrozoöid appeared. In one case there were three pre-differentiational divisions.

Regulation from the terminal branch zoöid 1*x* following simple decapitation of the growing point occurred in a high percentage of the cases (see Tables I and II). As far as the various levels along the primary axis were explored, the cells of the first few branch generations exhibited relatively frequent regulative responses. For the small number of operations made at high levels on old colonies, the transfor-

mation of $1x$ into a new terminal macrozoöid occurred with about the same frequency and with as much dispatch as for earlier periods, i.e. lower levels on younger colonies. The metamorphosis of the activated microzoöid $1x$ at levels above E required no more than the average time necessary for differentiation of apical cells produced at lower levels. This appears to correlate with the more or less uniform rate of normal axial growth (Fauré-Fremiet, 1930). Likewise $2x^1$ zoöids on the various branches responded in the majority of trials. Zoöids of the $3x^1$ generation or later failed to regenerate above the mid-region of the experimental colonies.

In order to test the responses of a branch cell of the third generation it was necessary to cut away the newer branches which had formed above it along the main axis. In tests of the fourth or later branch generations, a relatively large part of the colony had to be removed.

Compound Cuts.—When the terminal macrozoöid *and* the terminal cell of the last branch were removed, the terminal cell of the second preceding branch was frequently induced to differentiate into a new terminal macrozoöid. This particular relationship obtained for a limited number of successive branches and even then was unpredictable. For instance, the terminal macrozoöid was sometimes produced by the sub-terminal (a secondary) microzoöid of the newest branch despite the presence of a healthy terminal cell on the penultimate branch. In rare cases the latter assumed the regulative function in the presence of a complete uninjured branch between it and the cut-off.

The number of regenerates obtained from the sub-terminal branch zoöids is given in Table II. Microzoöids of the order $2x^2$ responded up to the level of branch I, whereas sub-terminal zoöids of the fourth generation ($4x^2$) did not respond at all. It is also certain that some colonies did not regenerate from either the sub-terminal zoöid of the first branch below the cut or from the terminal zoöid of the next adjacent branch. These branches continued to develop in a normal fashion for one or more generations without attempting to produce a new terminal macrozoöid.

The reactions of the older segments of well-developed colonies were tested by means of extensive "cut-backs," colonies cut off at some more basal internode. The data obtained (Table II) suggest an inverse relation between the number of regulative responses and the age of the activated zoöids, i.e. the frequency of responses diminishes as the number of lateral generations increases. The frequency of negative cases even in younger generations increased in the high levels, which is probably an expression of the fewer generations required to bring the more distal branches to full development. The

same data arranged according to amount of colony removed (Table I) show that basal branches may differentiate a new terminal macrozoöid after as many as seven branches plus the apical zoöid are cut away.

The cut-back experiments were successful only as regards the demonstration of initial reactivity to surgical alterations. The cases in Tables I and II where relatively large portions of the colonies were removed show only that the prospective values of certain zoöids along branch axes may or may not be modified, depending upon the amount of colony dissected away. The capacity of the responding zoöids for sustained growth is not known because all of the colonies cut back four or more branches had to be discarded before the regenerate attained full growth. Indeed, some were maintained under experimental conditions only long enough to produce a new apical cell. The chief difficulty is referable to the fact that the basal branches were the first to be attacked by vegetable growths propagating over the surface of the slide. The affected basal zoöids were shed before the colonies reached maturity. For this reason the zoöids of branch *A* on colonies with eight or more branches were usually unsuitable for testing. The age of the colony at the time of operation plus the additional time required for the differentiation of one of its zoöids gave to the parasites an advantage that was too frequently fatal to the experiment.

Regulation from the Axial Microzoöid Series.—Although nearly 9 per cent of the regenerates sprang from the axial microzoöid (1*X*) series, their regulative behavior was capricious and could not be induced at will. One of the most striking facts in this connection was the origin of new growths from 1*X* or descendants on complete, uninjured branches (Fig. 3). One originated from the third branch below the cut. Deliberate attempts to activate a given axial zoöid by eliminating all other zoöids on the branch resulted in (*a*) no further developmental activity, or (*b*) regeneration from some zoöid on the next lower branch. A three-cell colony which was trimmed down to a single cell, the axial microzoöid 1*A*, remained without further change for 165.5 hours; its contractile and feeding responses appeared to be normal for the entire period of observation. Similarly, the corresponding zoöids on branches *E*, *L*, *M*, and *P* of other colonies were tested without avail. In each case the regenerate developed from zoöids on the next lower branch and is so recorded. On the other hand, it has been demonstrated that the axial microzoöids are capable of regenerating (see 1*X* and 1*X*[1] in Table II).

The protocols show that 1*X* or some of its descendants carry terminal macrozoöid potencies. In some of these cases the regenerates

formed directly from $1X$ without leaving ciliospore-producing cells on the activated branch. The others regulated after $1X^1$ and $1X^2$ were formed; $1X^1$ gave rise to the new terminal macrozoöid while $1X^2$ produced one or, after division, two typical ciliospores.

Regulation after Successive Cuts.—The successive operations were of two general classes: (*a*) progressive, in which the second and third

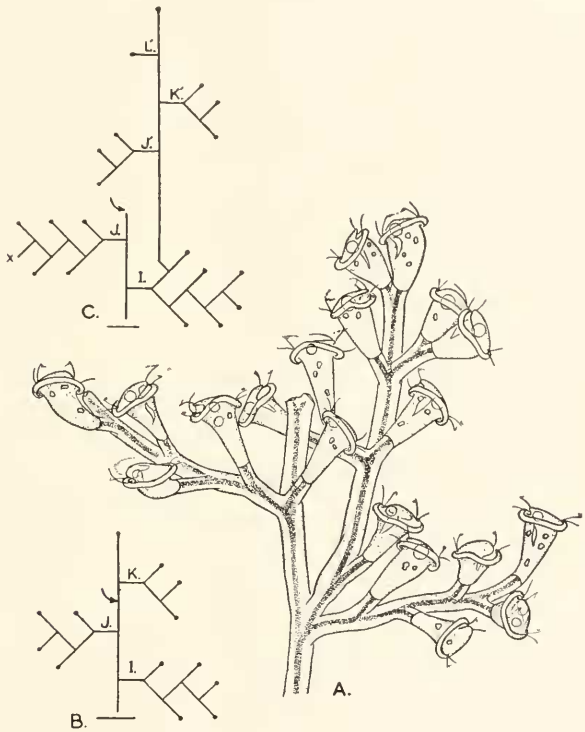

Fig. 3. *A*. Drawing of colony $3IJ$-2 approximately 72 hours after cutting. In this case the regenerate was produced by the axial microzoöid on the intact branch *I*. Axial microzoöid $1I$ divided before the new terminal macrozoöid differentiated: $1I^1$ regulated, leaving $1I^2$ near the base of the new axis. An increase in diameter of the new axis is evident near its junction with the original colony axis. × 250.

B. Condition of the apical end of the colony before cutting at the point indicated by the arrow.

C. Schematic representation of the resulting growth at the time the drawing was made.

cuts removed regenerated parts distal to the preceding operation (Fig. 4); and (*b*) regressive, where the entire regenerate plus additional parts of the original colony were cut off. In the first group the second and third regenerates were themselves products of regenerated segments. Those of the second group developed from some more basal

cell of the original colony. In this respect they were similar to the extensive cut-back types and subject to the same technical limitations.

The regulative activity of the zoöids of the second and third order regenerates in progressively cut colonies was similar in most respects to that evoked after simple cut-offs. In each of the nine cases studied the same morphological pattern and, as far as the limited number of cases permit judgment, similar developmental rates obtained after the second and third operations.

Incomplete Section of the Stalk.—In a few cases out of many trials a local injury to the neuro-muscular cord was effected without destroying the continuity of the cortical hyaline stalk substance. A re-

FIG. 4. *A*. Development after two successive operations (54 hours after the first cut). The original axis lies on the right. The severed peduncle of the regenerated terminal macrozoöid may be seen near the base of the second regenerate. Axial microzoöid 1*C* was badly parasitized; it dropped away soon after the drawing was made. × 250.

B. Schematic representation of *A*. The cuts are indicated by arrows. *Parasitized zoöid.

quisite degree of compression between the needles caused the cord to break down into a series of irregular protoplasmic droplets, some of which appeared to be independent of any attenuated membranous connectives.

Several interesting facts were brought out by this type of operation. The severed part of the neuro-muscular cord did not recover from the injury, i.e. the structural or functional continuity between the separated parts was not re-established. After the injury there was no subsequent degeneration of the cord in either proximal or distal parts. The functional unity of the whole was permanently impaired. The

proximal and distal parts contracted independently of each other and, as established in one case, the distal portion continued to grow and differentiate in the manner of an intact colony, whereas the proximal part regenerated a new primary axis from a zoöid below the injury (Fig. 5).

More substantial data are obviously required before definite conclusions can be drawn. Nevertheless these results do suggest interesting possibilities for further study. A thoroughgoing investigation of the contractile, transportative, and transmissive properties of the neuro-muscular cord may lead to a further elucidation of coördinating factors in colony formation. At least here is a clear indication that, whatever the physico-chemical nature of the integrative factors, they are probably mediated through the substance of the cord.

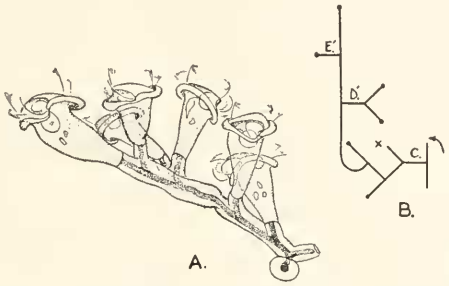

FIG. 5. *A*. Branch *C* of colony 9*B*4 56 hours after injury to the neuro-muscular cord (drawn from above). The original colony of six branches was pinched in the mid-region, isolating *ABC* from *DEF* plus the terminal macrozoöid. The terminal cell on branch *C* ($3c^1$) at the time of the operation differentiated into a new terminal macrozoöid which produced two new branches as shown. Note the increased diameter of the stalk just lateral to the second zoöid. This marks the position of the microzoöid $3c^1$ at the time of injury. × 250.

B. Schematic representation of branch *C* as drawn. Axial microzoöid 1*C* was accidentally cut away from the position marked (*x*).

Several completely isolated fragments were followed for a time by transferring every few hours to fresh filtered sea water. Nothing of unusual interest occurred in their development. Growth, differentiation, and regulation in progress at the time of cutting continued as before for the few generations that were followed. They did not re-attach to the substrate but developed as free-swimming fragments. Their growth capacities or the minimum size necessary for survival were not investigated.

Differentiation in Regenerated Parts.—The regenerates formed on decapitated colonies, after single or successive operations, are capable of producing any of the six types of heteromorphic zoöids previously described (Summers, 1938). According to the data compiled from

77 protocols the type, number, and distribution of zoöids on the regenerated parts compare favorably with the control colonies. The regenerates consisted of a new terminal macrozoöid, varying numbers of common microzoöids, a terminal cell at the tip of each branch, and one or more potential ciliospores, depending upon the degree of development following an operation. Macro- and microgamonts likewise differentiated on regulating parts with about the same frequency and vertical distribution as for corresponding regions of normal gamont-producing colonies. Regenerates sometimes differed from the controls in respect to the branch generation involved in the production of ciliospores and microgamonts. In normal colonies the ciliospores developed not earlier than the $1X$ generation and the microgamonts only from $2x^1$ or succeeding generations. On the regenerates one or the other of these two types of migrating zoöids frequently developed from the initial branch zoöid (Fig. 6*B*). This tendency towards

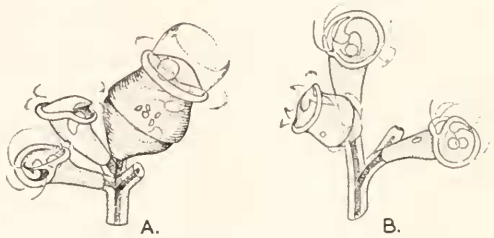

FIG. 6. *A*. Terminal macrozoöid 13′ (above branch *M*) differentiating into a ciliospore 204 hours after cutting. The colony was sectioned between branches *E* and *F*. × 250.

B. Metamorphosis of the median microzoöid *S*′ into a microgamont. The drawing was made 127 hours after regulation from the axial microzoöid 1*O*. × 250.

earlier differentiation obtained not only in the young regenerates on immature colonies but also in those derived from older (basal) zoöids of nearly mature colonies.

Another noticeable deviation from the established norm was the occasional metamorphosis of the terminal macrozoöid into a migratory zoöid, either ciliospore or microgamont (Fig. 6*A*), thus bringing to a close the growth along that particular axis. The terminal macrozoöid of the regenerate may differentiate directly into any of the three reproductive zoöids: microgamont, macrogamont, or ciliospore. Fauré-Fremiet (1930) reported the formation of the latter type in rare instances during the normal development of this species but the process was unaccompanied by the endomictic process which he described for normal ciliospore development.

While studying conjugation in *Zoöthamnium arbuscula*, Furssenko

(1929) observed that a local injury to one of the main branches affected the zoöids distal to the region of injury, inducing many of the microzoöids to metamorphose into microgamonts ("microconjugants"). Similar effects on the whole colony were induced by unfavorable environmental conditions, e.g. inanition or lack of oxygen. He did not observe a regulatory response subsequent to the injury.

Regulation after Conjugation.—From the following fragmentary account of the growth activity manifest in conjugating colonies it is at once clear that this aspect of development alone constitutes a lengthy research problem. Only ten of the colonies whose lineage was being followed happened to conjugate so that a detailed analysis

FIG. 7. A colony several days after the onset of conjugation at the level of branch *N*. The ex-conjugant divided into a cluster of large zoöids, one of which differentiated into a new *T.M.* whose further development prolonged the main axis. The exact lineage of these cells is not known. × 250.

of the process is not immediately available. Much of that which follows is based upon conjugants observed among the adventitious growths on the culture slides whose histories are but imperfectly known. Conjugation is introduced here because it affords one clue to qualitatively different physiological relations between the apical zoöid, the conjugant in this case, and a large area of the subordinate regions of the colony.

So far the results obtained from regulation experiments bespeak a regular functional correlation between the single cell in the apical position and the zoöids in sub-adjacent regions, such that the latter

are subservient to the former. Potentialities known to be present in zoöids of a lower order are presumably held in abeyance by the apical influence. Cutting away the apical region evokes a response in some zoöid in a subordinate but adjacent region. The response is a differentiation of another apical zoöid whose relations with the whole are seemingly homologous with those of the original apical cell. There may be a time between decapitation and subsequent regulation when the apical cell influences are altogether absent, yet the interim is not sufficiently great to seriously modify the observable growth phenomena. It is noteworthy that subordinate branches attain about the same end-point of lateral growth in control and decapitated colonies.

As stated in the previous work (Summers, 1938), the macrogamonts were observed only in the terminal macrozoöid positions 3 to 24 along the primary axis. The fusion of gamonts invariably brought axial development temporarily to a close some 12 to 13 hours after the last mitosis.

The conjugant remained quiescent for periods of about four days, then divided into two moderately large zoöids. One of the two ex-conjugants assumed the form of a terminal macrozoöid and resumed axial development after the four-day interruption. The fate of the sister ex-conjugant is a matter for conjecture at present; some disappeared from the colonies between observational periods while others divided into clusters of from two to seven large ciliospore-like zoöids at the base of the new axis (Fig. 7). The histories of these are likewise unknown. Apparently they do not propagate additional axes while associated with the parent colony. The development of ciliospores from some of the ex-conjugants in *Zoöthamnium arbuscula* (Furssenko, 1929) is suggestive, however.

The point to be made relates primarily to the behavior of the colony as a whole following the conjugation process. Prior to the completion of conjugation and continuing thereafter a new growth phenomenon appeared. The first three or four branches below the presiding conjugant developed out of all proportion to the average expectations (Fig. 8). The number of branch generations was in some instances greater than twice that of corresponding branches in controls. Moreover, many of the common lateral or secondary zoöids were activated to divide one or more times, originating second order branches which, in turn, sometimes produced tertiary branches. In this way each of the first few branches below the conjugant level grew almost as individual colonies. The greatest lateral growth effect obtained nearest the conjugant and diminished basally as a gradient. The normal tendency toward a pyramidal colony pattern was thereby

reversed in the environs of the conjugant. More precise information regarding growth intensity and capacity factors in both the ex-conjugant strain and the subordinate branches awaits further investigation.

Discussion

One of the most important consequences of the work is the demonstration of qualitatively different physiological relations between

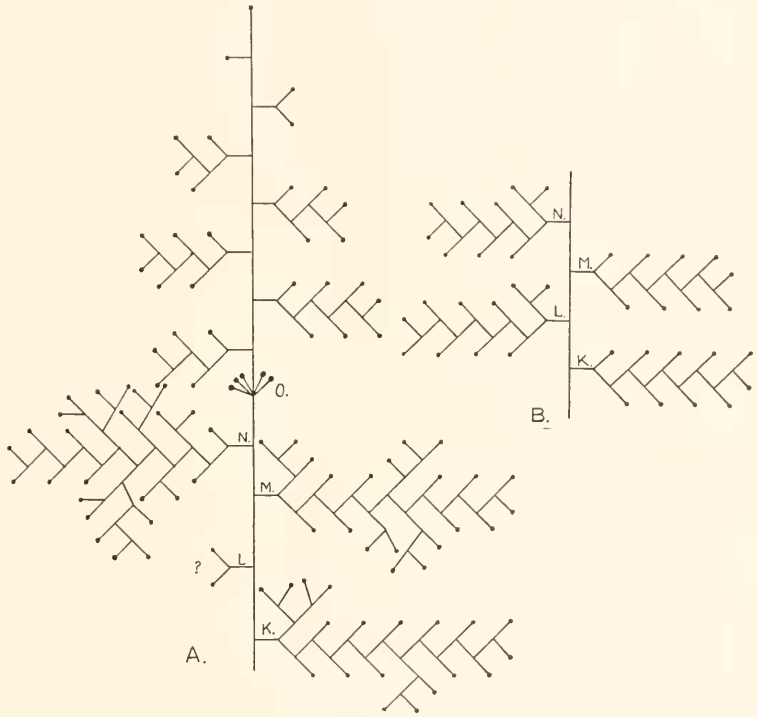

Fig. 8. *A*. Schematic representation of protocol 19*oe* to show the disproportionate development of branches *K*, *M*, and *N*. The lineage of branch *L* could not be deciphered. One of the ex-conjugant zoöids at *O* produced the apical growth illustrated in the diagram.

B. The corresponding portion of the largest of the 70 control colonies.

spatially separated cells. Under normal conditions a specific pattern unfolds. When an apical region of a colony is cut away some zoöid of a lower order, one whose complete developmental possibilities are otherwise never expressed, assumes the dominant generative functions, and the characteristic pattern perseveres. So far these results are intelligible in terms of what Child (1929) calls physiological correlation: the relations of dominance or control and subordination between

parts. He concludes "that dominance and subordination depend primarily on quantitative, rather than specific differences in physiological condition and that they represent a certain aspect of a physiological gradient." In *Zoöthamnium alternans* the transformation of a terminal macrozoöid into an ex-conjugant initiates an entirely different developmental phase which gives another clue to the general nature of apical control. Four days after the fusion of gamonts the normal growth relations of a varying number of branches below the conjugant level are upset in a rather remarkable way. Each of the three or four adjacent branches develops out of all proportion to the normal expectations. The precocious mitotic activity produces secondary and even tertiary axes on the affected parts. This unusual phenomenon does not occur when the terminal macrozoöid is present *or* when it is absent; it is effected by some new quality in the coördinating mechanism arising in consequence of conjugation activities in one particular cell—the apical cell.

The effects obtained after decapitation and conjugation certainly suggest that the single cell in the apical position is responsible in a large measure for quantitative and qualitative regulation of the part-whole relationship and that the control varies with respect to the local activities of the parts.

There also seems to be a coördination between the cells on different branches. A new terminal macrozoöid arising from one of the branch cells exerts its influence from what was formerly considered to be a branch position. Perhaps branch-to-branch coördination also explains the stable activity of the variously placed cells in the interim before a new apical cell differentiates; only one of the several possible zoöids regulates. The control of a terminal branch cell over the cells on its own branch can be interpreted in a similar way. As long as the terminal cell presides over a branch strain its immediate relatives remain quiescent. If it is destroyed, however, the sister at the subterminal position assumes the functional rôle of the lost cell.

A rather wide variation in the degree of regional correlation is suggested by those instances where a new terminal macrozoöid arises, not on the first branch as usual, but on the second or even third branch below the operated level.

The axial microzoöids on every branch do not differentiate into ciliospores. Loci of metamorphosing zoöids occur on about every third or fourth branch. The prospective value of a single axial microzoöid (1*X*) is predictable at a relatively early period by a marked growth in size. The growth may be taken as a criterion of at least a partial differentiation, to be completed a good many hours later

by a further increment in volume, modification of form, appearance of motile organelles, etc. A peculiar characteristic of these large cells is that they may differentiate directly into mature ciliospores or they may divide, giving origin to two zoöids of unequal size, both larger than any of the common types. The larger of the two matures first; the smaller grows to the size of its predecessor before metamorphosing or dividing again to produce two mature ciliospores in succession.

Regenerates sometimes arise from either those axial microzoöids which are, to all appearances, not predestined to metamorphose, or from those already partly differentiated. In the latter a potency or potencies in the process of expression apparently can be altered or superseded by others whose "urgency" toward expression is greater. The directional change in the process is referable to stimuli arising from the altered colonial organization. This is but another bit of evidence to the effect that cellular organization is dynamic and labile at certain periods and that changes going on within the cell which lead to recognizable morphogenetic characteristics are not necessarily irreversibly determined in direction. Many cases are known among the Protozoa where extrinsic or intrinsic factors lead to periods of reorganization, varying in considerable degree for the different groups and at different periods in the life cycle. In *Zoöthamnium alternans* we have a case where the re-direction of morphogenetic processes can be traced to an extrinsic cause: cutting the colony in the near vicinity of the cell subsequently affected.

Several significant problems arise in connection with the variable response of the axial microzoöids. Why do regenerates sometimes arise from axial microzoöids (1*X* or descendants) when as a rule the new growths are derived from the terminal branch cells? Attempts to induce regulation from 1*X* cells by trimming away all other cells on the branch gave no positive or predictable results. Until the question is investigated further in *Zoöthamnium* we can only interpret the variable behavior in terms of other work. Some of the merotomy experiments on other protozoa are suggestive (Calkins, 1911*a*, *b;* Peebles, 1912; Young, 1922; Dembowska, 1926; Taylor, 1928; and others). With respect to regenerative capacity these investigators were able to demonstrate progressive physiological changes in the cellular organization during the inter-mitotic period. Fragments cut at successive intervals after fission gave an increasingly high percentage of perfect regenerates. In *Uronychia* (Calkins, 1911*a*) even an emicronucleated fragment regenerated when cut immediately before the onset of fission. But in nearly all of the different forms studied the regenerative tendency disappeared sometime during the division process.

Another line of investigation summarized by Calkins (1934) and Summers (1935) demonstrates the cytological changes in cell organelles coincident with the division process. The resorption of old and the reappearance of new motor organelles, macronuclear reorganization, etc. suggest a brief period of cellular de-differentiation. There are probably analogous processes of alternating differentiation and de-differentiation in the history of the individual zoöids in *Zoöthamnium*. Time may be one of the important factors in individual cell behavior in relation to the balance between extrinsic coördinating influences and the aggregate of intracellular activities. That is to say, the intracellular activities of a *Zoöthamnium* cell may lead to the "fixation" of specific potencies at some critical period after cell division or, conversely, a cell may be more susceptible to the coördinating influences during or immediately after a division process. The axial microzoöid, for example, divides in from 20 to 70 hours after its derivation from the initial branch cell, whereas the terminal cell on the branch generally divides at intervals of about 12 hours. If a decapitation is made at a moment when the axial microzoöid is in some phase of divisional reorganization and the terminal cell in a more stable condition, the former instead of the latter may be activated or excited to prolonged generative activity. The supposition should be tested by a series of accurately timed operations above some particular branch.

An explanation of morphogenetic processes in *Zoöthamnium alternans* in terms of embryonic segregation at the time of division has already been attempted by Fauré-Fremiet (1930). In order to outline several points for discussion it is essential to review briefly his cytological analysis of normal development in this species. First, as regards the early axial divisions, the first three generations along the primary axis are unequal divisions. The inequality of the resulting daughters is reflected in the assortment of macronuclear material; each time the zoöids which remain in the terminal position (*TM*. 1, 2, 3) receive a larger portion of the macronuclei than the smaller branch microzoöids (*A*, *B*, *C*). On the supposition that the enlarged end of the macronucleus apportioned to the terminal macrozoöids represents a kind of segregation of chromatin material, these three unequal divisions are described by Fauré-Fremiet as qualitatively and quantitatively differential divisions. Beginning with the fourth division (division of *TM*. 3) the extremities of the dividing macronuclei in all later axial divisions are similar in size but in each instance a bit more of the finely striated mid-portion of the macronucleus is received by the zoöid remaining in the terminal position. All of these later divisions are characterized as quantitatively differential only. With

respect to the branch generations, all divisions along branches *A*, *B*, and *C* are similar and almost equal. The initial zoöids of subsequently produced branches (*D*, *E*, *F*, etc.) undergo qualitatively differential divisions: the axial microzoöids (1*D*, 1*E*, 1*F*, etc.) receive a greater share of the macronuclei than their lateral sisters (1*d*, 1*e*, 1*f*, etc.) although the cytoplasm in each case is distributed equally The axial microzoöids (1*D*, 1*E*, 1*F*, etc.) represent the ciliospore-producing members of the colony. They undergo marked growth in size accompanied by a disintegration and reconstitution of the macronuclei which, although not described in detail, is characterized as an endomictic process. Fission in the lateral sisters (1*d*, 1*e*, 1*f*, etc.) is of no further interest cytologically; these zoöids constitute the main branch strains.

From the foregoing description it follows that differential division occurs at two points in the formation of all branches above *C*. To illustrate (Fig. 9): *D* receives less cytoplasm and macronucleus than

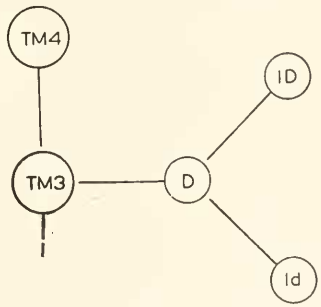

FIG. 9.

TM. 4, but the macronucleus in both resulting zoöids is qualitatively similar. The division is therefore quantitatively differential. When the initial branch cell *D* divides its cytoplasm is distributed equally but the macronucleus is assorted differentially because a thickened granular part goes wholly to the axial microzoöid 1*D*. The division is therefore qualitatively differential.

According to Fauré-Fremiet, "It appears clearly then that during the growth of a colony of *Zoöthamnium alternans* the two cells resulting from a division of one initial cell are never equivalent as to their 'potentialities.'" Also, "The character of the differential divisions on the main strain seems to determine the individual's differentiation of the colony; this differentiation depends not only on the individual's size, but also upon its physiological potencies." The differential divisions also appear to determine the characteristic features of the

median individuals and of the microzoöids. The latter have a limited power of growth and multiplication. Median microzoöids on branches *A*, *B*, and *C* remain as common nutritive zoöids, whereas the corresponding zoöids or their descendants on branches above *C* may undergo considerable growth and metamorphose into ciliospores.

Is it to be inferred from this analysis that quantitatively differential divisions determine (restrict) the subsequent power of division in branch strains and, further, that qualitatively differential divisions effect a segregation of potencies for ciliospore formation?

Regarding the first inference, all alternating median microzoöids, initial branch cells, are the lesser products of quantitatively differential divisions; they can divide only so many times, according to the number of generations normally produced on the branches of which they represent the beginning. While there is no doubt about the inequality of the cytoplasmic distribution between terminal macrozoöids and initial branch cells for the first ten generations or more, the inequality diminishes beyond this point until the two daughters are no longer differential as regards volume of cytoplasm. An equality may be achieved as early as the tenth and not later than the twentieth axial generation. Then what of those colonies that developed eight to fifteen generations beyond the twentieth node with similar axial-lateral relations? Another point to be made pertains to the regulative capacity of the branch zoöids. Those distributed in alternate positions along a branch axis seldom divide further so long as the integrity of the whole colony is preserved. When an apical piece is cut away from the colony some one of the more lateral zoöids on the remaining portion is capable of assuming the principal generative functions for relatively long periods of time. This behavior is difficult to interpret on the assumption that mitotic or "growth" potentialities are conditioned at either or both of the first two generations on the regulating branch.

The second inference may be challenged upon the grounds that segregation of ciliospore potencies does not occur at the specified division. Fauré-Fremiet adduces cytological evidence of segregation at the first three axial divisions and thereafter at the division of the initial branch zoöids. The latter is the fission at which the ciliospore-forming zoöids are separated from the main branch strain. In the light of newer findings, the restriction of ciliospore formation to 1*D*, 1*E*, 1*F*, etc. can be questioned. Ciliospores were observed to develop from axial microzoöids on the second and third branches, and also from both daughters of the fifth and tenth generations on branches *D* and *H* respectively of control colonies. Moreover, ciliospores oc-

curred on the regenerate in nearly every case of regulation from branch zoöids lateral to the supposed differential division provided, of course, that they were maintained for a sufficient length of time. The final bit of evidence comes from the demonstrated regulative capacity of the axial microzoöids (1X) on some of the operated colonies. When activated these were able to regenerate comparatively large sections of colony upon which all classes of zoöids except gamonts appeared.

The spatial relationship of cells should not be minimized as an important determining factor in an organism whose cells are so characteristically placed. The importance of this factor in development cannot be properly valuated until the general physico-chemical nature of the integrating mechanism and the medium through which it operates, presumably the neuro-muscular cord, are more fully understood. It is fairly certain, however, that it is not a specific factor, for in normal uncut colonies ciliospores occasionally develop in odd positions, and the microgamonts are apt to differentiate from the common zoöids at almost any position lateral to the axial microzoöids. Of related interest is the work of Buchanan (1927) on the flatworm *Phagocata*. The region from which a piece is taken with reference to the mouth of the intact worm is of no significance in determining the location of the mouth in the regenerate. Seyd (1935), on the other hand, reported a definite degree of regional specificity in the regeneration of a new mouth in *Spirostomum;* mouths in abnormal positions in the cut organisms degenerated and new ones formed at the correct locations.

The conjugation processes in *Zoöthamnium arbuscula* (Furssenko, 1929) and *Z. alternans* do not differ in essential detail. In the former the zoöids at the terminal position on each of the two primary axes (A_e and B_e) becomes the "macroconjugants." Until metagamic divisions occur, further growth along these axes is arrested. Two metagamic divisions of the conjugant result in a cluster of four large zoöids, two of which (A_2 and C_2) metamorphose directly into macrozoöids (ciliospores). The remaining two (B_2 and D_1) divide again, each giving rise to another macrozoöid and a stem cell. A stem cell produces an additional macrozoöid and a new (ex-conjugant) axis. A single conjugant therefore produces two ex-conjugant axes and from four to six successively produced macrozoöids.

The behavior of small lateral branches from the main axis at nodes basal to the conjugant in *Z. arbuscula* compares favorably with the precocious development of subordinate branches on a conjugating colony of *Z. alternans*. One or two of the small lateral branches (Seitenätschen) below the conjugant develop to the proportions of

regular main branches. In this fashion normal colonies with seven main axes are transformed, after conjugation, into colonies with nine to eleven chief axes. Furssenko refers to the new growths as "compensation" branches (Ersatzzweigen). It is his belief that the enormous growth of the macrozoöids occurs at the expense of food obtained from nearby microzoöids and, similarly, the compensation branches are destined to supply the energy needs of the ex-conjugant derivatives, i.e. the five or six clustered macrozoöids and the new ex-conjugant axes.

The chief support of Furssenko's hypothesis that ex-conjugant generations develop at the expense of adjacent regions comes from two observations: (*a*) the ex-conjugants themselves are not active feeders, and (*b*) the macrozoöids in neighboring regions either fail to mature or they divide to form common zoöids. The idea presupposes a mobilization and free transport of nourishment to regions active in development.

The observations on *Zoöthamnium alternans* are not in any way contrary to a possible rôle of the stalk in transportative phenomena. It is also quite likely, although yet to be proved, that nutrient materials are utilized by some zoöids at the expense of adjacent ones; this may be a cause contributing to growth inhibition or differentiation in nearby cells. Nevertheless it does appear that the precocious development of subordinate branches in a conjugating colony of *Z. alternans* is not primarily directed toward nutritional ends. In the first place, the actively developing apices of branch strains have energy requirements which, when taken as a whole, undoubtedly exceed the demands of the single ex-conjugant or its first few non-feeding descendants. The flux would therefore be directed away from the conjugant node. Secondly, in nearly every instance recorded the unusual development of the subordinate branches was well under way before the first post-conjugant division occurred. It is problematical whether or not the change in food requirements coincident with the transformation of a terminal macrozoöid into an ex-conjugant is sufficiently great to account for the relatively far-reaching alteration of the growth pattern.

Summary

1. *Zoöthamnium alternans* is a colonial protozoan of a rather special type whose constituent cells collectively possess in some degree many of the attributes of an integrated organism. Some of the integrating factors can be described in general terms from the work undertaken on form regulation.

2. When the apical cell of the primary axis is dissected away from a developing colony, a cell on some inferior branch, usually the first

below the cut, will differentiate into a new apical cell. The geographical limits within which positive regulative responses occur are given in the text.

3. Development of a colony continues from the newly differentiated apical cell. The structural and developmental characteristics of the normal colony persevere in the regenerated portion.

4. Evidence is presented to the effect that zoöids retain, for a time at least, greater developmental potentialities than are actually expressed when they comprise a part of the intact colony.

5. Under varying physiological conditions in the apical region of a colony, the coördinating influences exerted upon the mitotic activity of neighboring zoöids may be inhibitory (as shown by the responses evoked after decapitation) or excitatory (when the terminal macrozoöid is transformed into an ex-conjugant).

6. In the light of observations presented, the idea of dichotomous segregation or sifting out of potencies at fission is inadequate as an explanation of localization in this species. The experimental data do not confirm Fauré-Fremiet's cytological account of qualitatively differential divisions at specified division nodes on the branches.

7. There is cause to suspect that morphogenetic processes in particular zoöids of *Zoöthamnium alternans* (e.g. the presumptive ciliospores), once initiated and partly expressed in visible structure, can be conditioned or modified by cuts made in some neighboring region.

8. An hypothesis is offered to account for the origin of a regenerate from one or the other of several dissimilar cells of a branch strain. The explanation is based upon the factor of time in relation to the balance between extrinsic influences and the aggregate of intracellular metabolic activities by which potentialities are realized. The cells are thought to be more susceptible to external control during the reorganizational period of mitosis. There may be a critical time in cellular differentiation beyond which the intrinsic processes are not influenced by stimuli arising in some other part of the colony.

LITERATURE CITED

BUCHANAN, J. W., 1927. The spatial relations between developing structures. I. The position of the mouth in regenerating pieces of Phagocata gracilis (Leidy). *Jour. Exper. Zoöl.*, **49**: 69.

CALKINS, G. N., 1911*a*. Regeneration and cell division in Uronychia. *Jour. Exper. Zoöl.*, **10**: 95.

CALKINS, G. N., 1911*b*. Effects produced by cutting Paramecium cells. *Biol. Bull.*, **21**: 36.

CALKINS, G. N., 1934. Factors controlling longevity in protozoan protoplasm. *Biol. Bull.*, **67**: 410.

Child, C. M., 1929. Physiological dominance and physiological isolation in development and reconstitution. *Roux' Archiv. f. entw.-mech.*, **117**: 21.

Dembowska, W. S., 1926. Studies on the regeneration of Protozoa. II. Regeneration of the ciliary apparatus in some marine Hypotricha. *Jour. Exper. Zoöl.*, **43**: 485.

Fauré-Fremiet, E., 1930. Growth and differentiation of the colonies of Zoöthamnium alternans (Clap. and Lachm.). *Biol. Bull.*, **58**: 28.

Furssenko, A., 1929. Lebenscyclus und Morphologie von Zoöthamnium arbuscula Ehrenberg. *Arch. f. Protist.*, **67**: 376.

Lillie, F. R., 1929. Embryonic segregation and its rôle in the life history. *Roux' Archiv. f. entw.-mech.*, **118**: 499.

Peebles, F., 1912. Regeneration and regulation in Paramecium caudatum. *Biol. Bull.*, **23**: 154.

Peebles, F., 1931. Some growth-regulating factors in Tubularia. *Physiol. Zoöl.*, **4**: 1.

Seyd, E. L., 1935. Studies on the regulation of Spirostomum ambiguum Ehrbg. *Arch. f. Protist.*, **86**: 454.

Summers, F. M., 1935. The division and reorganization of the macronuclei of Aspidisca lynceus Müller, Diophrys appendiculata Stein, and Stylonychia pustulata Ehrbg. *Arch. f. Protist.*, **85**: 173.

Summers, F. M., 1938. Some aspects of normal development in the colonial ciliate Zoöthamnium alternans. *Biol. Bull.*, **74**: 116.

Taylor, C. V., 1928. Protoplasmic reorganization in Uronychia uncinata, sp. nov., during binary fission and regeneration. *Physiol. Zoöl.*, **1**: 1.

Weiss, Paul, 1935. The so-called organizer and the problem of organization in amphibian development. *Physiol. Rev.*, **15**: 639.

Wesenberg-Lund, C., 1925. Contributions to the biology of Zoöthamnium geniculatum Ayrton. *D. Kgl. Danske Vidensk. Selsk. Skr., naturv. og. math., Afd.*, 8. Raekke, **X**: 1.

Young, D. B., 1922. A contribution to the morphology and physiology of the genus Uronychia. *Jour. Exper. Zoöl.*, **36**: 353.